Sociobiology 65(1): 108-111 (March, 2018) Special Issue DOI: 10.13102/sociobiology.v65i1.2095

Sociobiology
An international journal on social insects

RESEARCH ARTICLE - TERMITES

# Suitable Light Regimes for Filming Termites in Laboratory Bioassays

YC Carvalho, LO Clemente, MP Guimarães, O DeSouza

Lab of Termitology, Department of Entomology, Federal University of Viçosa, Viçosa-MG, Brazil

**Article History**

**Edited by**
Gilberto M. M. Santos, UEFS, Brazil
Received 26 September 2017
Initial acceptance 06 January 2018
Final acceptance 06 January 2018
Publication date 30 March 2018

**Keywords**
*Cornitermes cumulans*, infrared, LED, Isoptera.

**Corresponding author**
Og de Souza
Lab of Termitology
Depto Entomology
Federal University of Viçosa
CEP 36570-900, Viçosa-MG, Brazil
E-Mail: og.souza@ufv.br

**Abstract**

Laboratory bioassays require strategies to minimize stress and keep animals alive as long as the test demands. Sometimes, however, experimental procedures seem notoriously stressful as, for instance, when exposing termites to the illumination needed for video recordings. Being a condition opposed to what termites naturally experience, light might easily stress such insects or might not affect them at all, as they are blind. Here we check for the effects of distinct light regimes on the survival of termites confined in a typical bioassay setup involving footage. The survival of Cornitermes cumulans (Termitidae: Syntermitinae) workers kept in the dark, or subjected to infrared or to cold white light was compared, finding no statistical difference in their survival in these three treatments. While pointing directions for further research on the reasons for such results, we conclude that video recordings of *C. cumulans* termite workers can be conducted under infrared or cold white LEDs, as these light regimes do not affect the survival of tested individuals.

## Introduction

Laboratory bioassays require methods to keep animals in conditions as close as possible to their natural ambiance, so that to minimize stress and keep individuals alive as long as the test demands. However, some experimental procedures do require unnatural, potentially stressful conditions, such as the illumination demanded for video recordings of termite behavior. After all, by living inside nests and galleries and foraging only inside tunnels, most termites are better thought as accustomed to dark than to light conditions. Illumination normally needed for video recordings would, hence, be a potential source of stress for these insects.

In fact, termite workers and soldiers – the castes normally used in bioassays – show some aversion to open air conditions, ducking to darker places when their mound is opened (Williams, 1959) or when exposed to incandescent light in experimental arenas (Park & Raina, 2005). Being eyeless, these termites would not be thought to be bothered by light, escaping to darken areas for reasons other than typical photophobia. Further studies, however, have shown that ultraviolet light percolating through the unpigmented cuticle of *Reticulitermes* spp. can harmfully react with an alkaloid norharmane present in their hemolymph, impairing these termites' survival (Siderhurst et al., 2006)

Whether or not unpigmented termites other than *Reticulitermes* would equally be harmed by UV-light exposure, remains to be tested. After all, norharmane is produced by endosymbionts (Siderhurst et al., 2005) whose species composition varies across termites (Ohkuma, 2008). It also remains to be tested whether other electromagnetic wavelengths could harm termites as UV-light does.

On the other hand, even species such as *Coptotermes formosanus*, presenting low norharmane amounts (Itakura et al., 2008), do show aversion to light (Park & Raina, 2005), reinforcing the idea of light as a potential source of stress, irrespective of norhamane content, at least for the so called "lower termites".

On their turn, many "higher termites", belonging to the family Termitidae, present unpigmented workers and cryptic habits similar to those of *Reticulitermes* and *Coptotermes*, being hence potentially affected by light exposure. Among

these, the Neotropical *Cornitermes cumulans*, would seem to deserve attention. Being easily captured from conspicuous and populous nests, this species large individuals allow video recording without macro lenses, and are hence an obvious choice for lab bioassays footage. Miramontes et al. (2014), for instance, studying patterns of spatial exploratory behavior in individual termites, video-recorded the movement of *C. cumulans* workers continuously for 5-6 hours. In order to accurately track every termite cartesian location during the assay, the arena was constantly illuminated by a cold white fluorescent lamp.

Here we intend to contribute to this type of study, testing the effects of distinct light regimes on the survival of *C. cumulans* workers confined in arenas, as typically done in many lab bioassays. Three light regimes were tested: no lights (*i.e.* darkness), infrared, and cold white lights. Infrared illumination is the closest to darkness an affordable camera could record whereas cold white illumination is suitable to footage by any type of camera. If *C. cumulans* is harmed by illumination (as in *Reticulitermes* affected by UV-lights), we expect their survival to differ between these light regimes, no difference in survival being found otherwise.

## Materials and Methods

### *Focal species*

*C. cumulans* live inside epigeous nests and feed on living and dead grasses and herbs, which they reach through subterranean tunnels, occasionally foraging under a fine layer of soil-sheeting (de Negret & Redford, 1982). Their mounds are abundant in grasslands in the Neotropics, and are found in pastures, monocultures, savannas (Araujo, 1970) and even in gardens in urban areas. This species builds mounds with a very hard outer shell of soil which surrounds a soft inner core of carton (fecal material, comminuted plant material and bits of soil) (de Negret & Redford, 1982). *C. cumulans* is a key species in grasslands, providing stable and predictable shelter for other termites, invertebrates, and vertebrates (Redford, 1984; Campbell et al., 2016).

### *Data collection*

The study was carried out in Viçosa, in the state of Minas Gerais State, Southeastern Brazil, located at 20º45' S 42º51' W, in the facilities of the Lab of Termitology, Federal University of Viçosa ('UFV' in Brazilian acronym). The experiment aimed to verify the effect of distinct illumination regimes on the survival of *C. cumulans* workers kept in Petri dishes in the lab. Termites were collected from colonies found in the UFV's *campus* on three occasions: 30 May, 2nd June and 5th June 2017. Fragments from three different nests were collected per date, totaling nine nests. Each nest fragment was taken to the lab, where 20 workers (third instar and beyond) were extracted to form an experimental unit confined in a closed Petri dish (59 mm internal diameter). A total of nine experimental units were formed, three for each illumination regime: (i) dark; (ii) cold white LED (OUROLUX™12W; color temperature: 6500K; luminous flux: 1200lm; luminous efficacy: 100lm/W); (iii) infrared light (ILS, OSLON IR 1 PowerStar IR Star LED, peak wavelength: 850nm, radiant flux 1070mW, radiance angle: ±45, 2-pin, SMD, using current=1.12A;voltage=15v). Experimental units were then taken to BOD incubator, under 25ºC±1, within which one of the three types of illumination regimes was set up. No food or water was provided in the Petri dishes.

Since group size can affect survival in termites, and this survival is associated with the spatial density termites are subjected to, we kept each experimental group at a density of 0.14 (the ratio between the total area occupied by termites and the total area of the arena's floor). This is within the range 0.10 to 0.19 considered to favor survival in termites (Miramontes & DeSouza, 1996; DeSouza et al., 2001; DeSouza & Miramontes, 2004)

Counting of dead and live individuals proceeded every hour in each Petri dish for the first 12 h after which it was halted for the next 8 hours. Then the hourly tally was resumed until all individuals were dead.

### *Data analysis*

Data were subjected to censored survival analysis under Weibull distribution, similar to what DeSouza et al. (2009) have done, using survival package in R (R Core Team, 2015). Survival analysis is the statistical analysis of data where the response of interest is the time, t, from a well-defined time origin to the occurrence of some given event (end-point) (Martinussen & Scheike, 2006). In our case, time origin is when we put the Petri dish inside the BOD incubator and the end-point is when we record the death of a termite worker in this arena.
The general model for this analysis follows the equation:

$$\log_e S(t) = \mu^{-\alpha} t^{\alpha}$$

Where $S(t)$ is the accumulated proportion of dead termites until time, $t$; the mean time μ is the time elapsed until 50% of termites are dead in a given treatment; and α is the shape parameter for the survival curve. When $\alpha=1$, the probability of a termite dying does not change as time elapses. If $\alpha<1$ this probability reduces as time elapses, the opposite happening when $\alpha>1$.

Statistical analysis was used to check whether the light regime would affect the mean time, μ, until termites are found dead in a given treatment. Under the null hypothesis, the mean time, μ, does not differ between light regimes, hence, time, t, alone explains S(t). That is, under the null hypothesis, termite deaths are not speeded up nor delayed by the light regime. Alternatively, if the light regime affects the time at which a termite dies, a typical μ can be calculated for each regime.

Modelling proceeded by building a full model with a single term, light regime, represented by a categorical variable with three levels: darkness, infrared light, and white light. Model simplification was performed removing this term from the full model and inspecting the consequent change

in deviance. In case of significant changes (*i.e.*, $P \leq 0.05$), the term would have been returned to the model to proceed further simplification amalgamating its levels.

## Results

*Cornitermes cumulans* termite workers confined in Petri dishes in a BOD incubator kept at 25ºC did not show any obvious quantitative changes in behavior along the whole experiment. Within each of these experimental arenas, termites circulated normally, stopping to interact or simply passing by when meeting a given conspecific. Interactions involved antennations, allogrooming, mouth-to-mouth contacts (presumably, oral trophallaxis), and occasional mouth-to-anus contacts (presumably, anal trophallaxis).

Survival curves presented a shape parameter $\alpha>1$ ($\alpha=3.369$), implying that mortality tended to be more expressive towards the end of the experiment. In fact, after 500 min had elapsed, more than 80% of the termites were still alive in all treatments. Mortality crossed the 50% threshold more than 1000 min after the beginning of the experiment (Fig 1).

Light intensity experienced by termites in the experimental units varied from 0 lux (darkness) to 5 lux (infrared) to 672 lux (white LED light). This has led to an average survival time of 1085 min for termites kept in the dark, 1065 min for termites kept under white LED light and 1102 min for termites exposed to infrared light. These timings and hence their respective light regimes, however, did not differ statistically (Table 1, $P=0.31$).

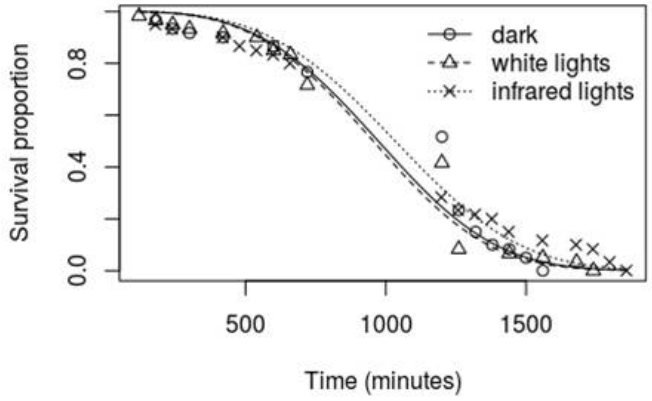


**Fig 1**. Survival of *C. cumulans* workers confined in Petri dishes in the dark, exposed to white lights, or exposed to infrared lights, in a BOD incubator kept at 25ºC. Curves do not differ statistically ($P=0.31$).

**Table 1**. Analysis of deviance for the effects of light regime on the survival of *C. cumulans* termite workers confined in Petri dishes. Three light regimes were applied continuously to the experimental arenas: darkness, infrared light, and cold white light. See Material & Methods for more detail. "LL" = log-likelihood.

| | Df | Deviance | Resid. Df | -2*LL | Pr (> $\chi^2$) |
|---|---|---|---|---|---|
| Null | | | 178.00 | 2660.90 | |
| Light regime | 2.00 | 2.31 | 176.00 | 2658.59 | 0.31 |

## Discussion

We did not observe any effect of the distinct light regimes on the mortality of *C. cumulans* workers in this work: such termites presented similar survival patterns when confined in darkness, under cold white light, or under infrared light (Fig 1, Table 1). While arising intriguing theoretical issues, these results have important practical connotations.

On the theoretical front, these results would evoke the question of why these termites, as opposed to *Reticulitermes*, were not harmed by the light regimes under test? Among plausible hypotheses, one has to consider the possibility that the light regime of highest intensity here applied (672 lux) was still below any harmful potential. This hypothesis, still to be tested, gains significance from the fact that daylight illuminance under bright sun attains not less than 100,000 lux, or 20,000 lux in the shade. An alternative hypothesis would point to the absence of phototoxic compounds in the hemolymph of *C. cumulans*. In order to answer it, one would have to inspect the presence of compounds such as norhamane, which is responsible for phototoxic effects in other termites (Siderhurst et al., 2006). Clearly, these hypotheses are beyond the aims of this work but we present them here in an attempt to sign the research avenues they could open.

On the practical front, our results validate the use of infrared and cold white lights in studies that demand video recording with *C. cumulans*, as none of these light regimes affected workers survival. Being closest to dark that we can record with a video camera, infrared would seem the best choice for such assays. As a drawback, not any camera can record video under infrared, and those which can, are normally more expensive (albeit many are still within the affordable price range). Compared to infrared lights, cold white LEDs are cheaper and easier to find. Better still, all cameras can record under this type of light.

As a word of caution, it must be considered that our test regards survival only. Despite not observing any obvious behavioral changes while counting dead and alive individuals for our test, no quantitative behavioral parameter was inspected (and this is, incidentally, another possible development from the current work). We conclude, therefore, that video recordings of *C. cumulans* termite workers can be conducted under infrared or cold white LEDs, as these light regimes do not affect the survival of tested individuals.

## Acknowledgements

We thank Helder Hugo dos Santos for providing us the infrared lights used in this study. This work was partially supported by CNPq, Fapemig, Capes. YCC, LOC, MPG hold MSc grants from CAPES. ODS holds a CNPq fellowship (#307990/2017-6). This work is part of the IV SymTermes Community at https://zenodo.org/communities/symtermes/?page=1&size=20, where it is identified by DOI 10.5281/zenodo.894421. This is contribution # 71 from the Lab of Termitology at Federal Univ. of Viçosa, Brazil (http://www.isoptera.ufv.br).

**References**


Araujo, R.L. (1970). Termites of the Neotropical region. In K. Krishna & F.M. Weesner (Eds.), Biology of Termites, vol II (pp. 527–576). New York: Academic Press.

Campbell, C., Russo, L., Marins, A., DeSouza, O., Schönrogge, K., Mortensen, D., Tooker, J., Albert, R. & Shea, K. (2016). Top-down network analysis characterizes hidden termite-termite interactions. Ecology and Evolution. doi: 10.1002/ece3.2313.

de Negret, H.R.C. & Redford, K.H. (1982). The biology of nine termite species (Isoptera: Termitidae) from the cerrado of central Brazil. Psyche, 89: 81–106.

DeSouza, O., Araújo, A. & Reis-Jr, R. (2009). Trophic controls delaying foraging by termites: reasons for the ground being brown? Bulletin of Entomological Research, 99: 603–609.

DeSouza, O. & Miramontes, O. (2004). Non-asymptotic trends in the social facilitated survival of termites (Isoptera). Sociobiology, 44: 527–538.

DeSouza, O., Miramontes, O., Santos, C. & Bernardo, D. (2001). Social facilitation affecting tolerance to poisoning in termites (Insecta, Isoptera). Insectes Sociaux, 48: 21–24.

Itakura, S., Kawabata, S., Tanaka, H. & Enoki, A. (2008). Effect of norharmane in vitro on juvenile hormone epoxide hydrolase activity in the lower termite, *Reticulitermes speratus*. Journal of Insect Science, 8: 13.

Martinussen, T. & Scheike, T.H. (2006). Dynamic Regression Models for Survival Data. Statistics for Biology and Health. 470 pp., New York: Springer Science+Business Media, Inc.

Miramontes, O. & DeSouza, O. (1996). The nonlinear dynamics of survival and social facilitation in termites. Journal of Theoretical Biology, 181: 373–380.

Miramontes, O., DeSouza, O., Paiva, L.R., Marins, A. & Orozco, S. (2014). Lévy flights and self-similar exploratory behaviour of termite workers: beyond model fitting. PLoS One, 9: e111183. doi: 10.1371/journal.pone.0111183.

Ohkuma, M. (2008). Symbioses of flagellates and prokaryotes in the gut of lower termites. Trends in Microbiology, 16: 345–352. doi: 10.1016/j.tim.2008.04.004.

Park, Y. & Raina, A. (2005). Light sensitivity in workers and soldiers of the formosan subterranean termite, *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Sociobiology, 45: 367-376.

R Core Team (2015). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Redford, K.H. (1984). The termitaria of *Cornitermes cumulans* (Isoptera, Termitidae) and their role in determining a potential keystone species. Biotropica, (pp. 112–119).

Siderhurst, M.S., James, D.M. & Bjostad, L.B. (2006). Ultraviolet light induced autophototoxicity and negative phototaxis in *Reticulitermes* termites (Isoptera: Rhinotermitidae). Sociobiology, 48: 27–49.

Siderhurst, M.S., James, D.M., Blunt, T.D. & Bjostad, L.B. (2005). Endosymbiont biosynthesis of norharmane in *Reticulitermes* termites (Isoptera: Rhinotermitidae). Sociobiology, 45: 687–705.

Williams, R. (1959). Flight and colony foundation in two *Cubitermes* species (Isoptera: Termitidae). Insectes Sociaux, 6: 203–218.